CALIBRATION PROTOCOL
1/18/07

(1) PURPOSE.

The purpose of this protocol is to foster the generation of environmental laboratory data that are scientifically valid, defensible and of known and documented quality by providing uniform guidelines for calibration of analytical instruments.

(2) LABORATORY ANALYTICAL INSTRUMENTS.

(a) All personnel operating analytical instruments shall be trained in the theory, selection, appropriateness, verification, limitations, diagnostic measures, and correctives associated with each calibration model used in any instrument they operate and the training documentation maintained.

(b) All instruments shall be maintained, inspected, and cleaned.

(c) Analytical instruments that give suspect results shall be taken out of service and repaired. The functional and calibration status of those analytical instruments are checked before returning to service.

(3) INSTRUMENT CALIBRATION GENERAL PROVISIONS AND REQUIREMENTS.

(a) This protocol establishes requirements to be met, at a minimum, by laboratories. Laboratories shall follow the more stringent requirements if mandated by test methods or regulations.

(b) All analytical instruments shall be calibrated or their calibration verified before they are used to provide quantitative results.

(c) Responses for all calibration standards shall be obtained following defensible and ethical practices.

(4) INITIAL INSTRUMENT CALIBRATION.

(a) The details of initial instrument calibration procedures, including, calculations, integrations, acceptance criteria, and associated statistics shall be included or referenced in the test method standard operating procedure and are made available to analysts

(b) The laboratory shall select a calibration model that is appropriate for the expected behavior of the analytical instrument to be calibrated.

(c) The minimum number of standard concentrations selected to establish calibration shall be 3 except for:

1. Dissolved oxygen meters, which shall be calibrated against air-saturated water or water-saturated air at a known temperature and pressure, or by reference to a single aliquot of air-saturated water analyzed by the Winkler or iodometric method.
2. Ion selective electrodes and pH meters, the minimum number shall be 2.
3. Inductively coupled plasma emission spectrophotometers and inductively coupled plasma mass spectrometers, the minimum number shall be one,
4. Calibration models that are quadratic, the minimum shall be 5.
5. Calibration models that are cubic, the minimum shall be 7.

Note: Very few detectors or instruments in environmental chemistry exhibit a cubic response when operated properly and when calibrated within the concentration ranges associated with mandated test methods or regulations. Before selecting a cubic model, analysts should ascertain that the choice is not used to compensate for performing necessary maintenance or for exceeding the expected calibration range of an instrument.

(d) The concentration of the standards chosen to establish a calibration function shall cover the desired quantitation range at approximately equally spaced intervals or shall make explicit any inflection points associated with the calibration function. Laboratories reporting results at levels at or near the limit of detection (LOD) of an analysis shall include in initial calibrations a standard at a concentration near the limit of quantitation (LOQ) of the analysis.

(e) The selected algorithm or reduction technique shall be describable mathematically, and shall provide equations, coefficients, or parameters necessary to characterize the calibration function uniquely, unless an analytical instrument is tuned to conform to a universally accepted scientific law or scale.

1. The laboratory shall select the simplest algorithm or reduction technique that satisfies calibration acceptability criteria, or
2. The laboratory shall not choose a more complex algorithm or reduction technique to compensate for instrument saturation, insensitivity, or malfunction.
3. The laboratory may use weighted algorithms or reduction techniques, unless they are chosen to compensate for instrument saturation, insensitivity, or malfunction.
4. The laboratory shall not use reiterative reduction techniques or algorithms that force calibration functions through zero, unless the test method specifically allows this procedure.

Note: Forcing through zero reiteratively generates a null response for a zero standard that has a non-zero response, or adjusts calibration parameters to obtain a null response without analyzing a calibration blank. This paragraph does not prohibit the use of average response factors or automatic zeroing as part of an initial calibration, when methods, regulations, or covered programs allow those techniques.

(f) The laboratory shall establish acceptability criteria for initial calibrations.

1. When average response factors are used the relative standard deviation shall not exceed 20% unless an approved method allows a larger percentage.
2. The correlation coefficient shall be at least 0.995 for inorganic analytes and metals, and at least 0.990 for organic analytes, regardless of the type of regression used.

(g) The laboratory shall establish procedures for the treatment of calibration blanks, when a mandated method or an analytical technique requires the response of a calibration blank to be part of a calibration function.

(h) Initial instrument calibrations shall be verified with a second source standard (different from source used to prepare calibration standards) prior to the analysis of any samples. This is not required for instruments calibrated by tuned to conform to a universally accepted scientific law or scale.

(i) Unless otherwise required by regulation, method, or program, the acceptance criteria for second source verification shall be the same as the criteria required for continuing instrument calibration verification.

(j) Laboratories shall quantitate sample results only from initial instrument calibrations, unless otherwise allowed by regulation, method, or covered program.

(k) All quantitated sample results shall be bracketed by calibration standards as specified in this section.

1. When samples cannot be diluted and reanalyzed, sample results shall be reported with appropriate flags, qualifiers, or narrative warnings.
2. When sample concentrations are below that of the lowest standard in the calibration curve, the reported results must have appropriate flags, qualifiers, or narrative warnings.
3. Samples with concentrations higher than the highest standard in the calibration curve shall be diluted and reanalyzed.

Note: Samples analyzed by ICP and ICP-MS having responses at or above 90% of the upper limit of the analyte linear dynamic range (LDR) should be diluted and reanalyzed.

(l) For single-analyte standard, eliminating standard responses from calibration is allowed, only under the following conditions:

1. The minimum standard points required by the protocol are maintained.
2. The retained lowest concentration standard is at or below the limit of quantitation or reporting limit for the analyte.
3. The reporting limit or quantitation limit is adjusted to be at or above the retained lowest concentration standard.
4. The retained highest concentration standard defines the highest range for reporting sample results without having to dilute.
5. An eliminated bracketed standard from calibration shall be adequately documented for cause, statistical evaluation and corrective action

(m) For multi-analyte standards, eliminating standard responses from calibration is allowed only under the following conditions:

1. The minimum standard points required by the protocol are maintained.

2. The retained lowest concentration standard is at or below the limit of quantitation or reporting limit for the analyte.
3. The reporting limit or quantitation limit is adjusted to be at or above the retained lowest concentration standard.
4. The retained highest concentration standard defines the highest range for reporting sample results without having to dilute (except ICP/ICPMS).
5. An eliminated bracketed standard from calibration for all analytes must be adequately documented for cause, statistical evaluation and corrective action.

(n) An initial calibration is finalized when an analyst reviews all standard responses, performs any necessary and defensible manual integrations or adjustments, eliminates any responses when allowed by this protocol, and determines the calibration function meets established acceptance criteria.

(o) Once samples have been quantitated using a finalized calibration curve, a laboratory shall not change the model or calibration function without performing another initial calibration and reanalyzing the affected samples,

(p) Laboratories shall perform an initial calibration:
1. After instruments undergo non-routine maintenance,
2. When repeated use or other conditions change their expected behavior,
3. When the continuing calibration cannot be verified.

(q) Laboratories shall retain all the raw data necessary to reconstruct initial calibrations.

(5) CONTINUING INSTRUMENT CALIBRATION VERIFICATION

(a) When an initial instrument calibration is not performed on the day of analysis or with a batch of samples analyzed, the validity of the initial calibration shall be verified prior to quantitating samples by continuing calibration verification with each analytical batch and at least once on each analysis day.

(b) Standard operating procedures shall document the process for the continuing calibration verification, calculations needed, and any additional statistics. Laboratories may reference a cited procedure as long as the cited reference is available in the laboratory.

(c) Continuing calibration verification standards should be made from the same source as the primary standards unless the method allows the use of a second source.

Note: Using verification standards from a source different from the one used to establish an initial calibration may promote the impression that a calibration is valid when initial calibration verification might have failed when using a verification standard from the same source used to generate the initial calibration. This is likely to occur when a verification and a second source

standard show marked and opposite biases when quantitated against an established calibration.

(d) The number of continuing calibration verification standards needed to be analyzed shall be based on the type of calibration model selected, the reduction technique or algorithm used, and the number of standards used to calibrate the instrument.
   1. When an instrument is tuned with one calibration standard, using a universally accepted scientific law or scale, then at least one continuing calibration verification standard shall be analyzed at any concentration.
   2. When an instrument is tuned with two calibration standards, using a universally accepted scientific law or scale, then at least one continuing calibration verification standard shall be analyzed at a concentration between the two standards.
   3. At least one verification standard shall be analyzed when using average of responses, or response factor, a linear regression analysis, or a curve that obeys a linear model. The concentration of the verification standard may vary within the calibration range.
   4. At least two verification standards shall be analyzed when using a quadratic regression or a curve that obeys a quadratic model. The concentration of at least one verification standard should be near the point of inflection.
   5. At least three verification standards shall be analyzed when using a cubic regression or a curve that obeys a cubic model. The concentration of two of the verification standards should be near the points of inflection.

(e) When internal standards are used to quantitate samples, a continuing calibration verification standard shall be performed:
   1. At the beginning of each analytical run, unless a calibration curve has been generated prior to samples being analyzed on the same day.
   2. After each group of 20 samples or after of a 12-hour period, whichever comes first.

(f) When internal standards are <u>not</u> used to quantitate samples, a continuing calibration verification shall be performed:
   1. At the beginning of each analytical run, unless a calibration curve has been generated prior to samples being analyzed on the same day.
   2. After each group of 20 samples or after a 12-hour period, whichever comes first.
   3. At the end of each analytical run.

(g) Unless otherwise required by regulation, method, or program, the laboratory shall establish acceptance criteria for the continuing calibration verification as follows:
   1. Within 10% of the standard concentration for reportable inorganic analytes and metals.

2. Within 15% of the standard concentration for reportable organic analytes.

(h) When the continuing calibration verification is outside the acceptance criteria, the laboratory may reanalyze the continuing calibration verification that failed. If the results of the reanalyzed continuing calibration verification are outside acceptance criteria, the laboratory shall take corrective action. If two consecutive continuing calibration verifications are within criteria after the corrective action, the laboratory may proceed with the analysis. If the two consecutive continuing calibration verifications are outside the criteria, the laboratory shall perform an initial calibration.

(i) Samples associated with failed calibration verification shall be reanalyzed, unless the following conditions occur:

1. Calibration verification recoveries were higher than the acceptance criteria and there were no detected corresponding analytes in the samples and sample results are reported with appropriate qualifiers.
2. Calibration verification recoveries were higher than the acceptance criteria and sample results were below regulatory or decision limits and sample results are reported with the appropriate qualifiers.
3. Calibration verification recoveries were lower than the acceptance criteria and the corresponding analytes in the samples are above the regulatory or decision limits and sample results are reported with the appropriate qualifiers.
4. Affected samples have been consumed and the sample results are reported with appropriate qualifiers unless after consultation with a regulatory authority or client, either one determines that re-sampling is necessary.
5. The holding time for the samples has expired and the results are reported with appropriate qualifiers unless after consultation with a regulatory authority or client, either one determines that re-sampling is necessary.

(j) Laboratories shall retain all the raw data necessary to reconstruct continuing calibration verifications.